

An updated review of routine bacterial surveillance of platelet concentrates

**Scientific Seminar on Transfusion Medicine
jointly organized by the HK Red Cross Blood
Transfusion Service and the HK Association of
Blood Transfusion and Haematology**

22 November 2003

Background

- Transfusion transmitted infection vs blood safety in a climate of public confidence.
- Technologies have substantially improved blood viral safety.
- Bacterial sepsis is still a significant and universal problem - can result in significant rapid onset morbidities and even mortalities.



Frequency of Transfusion-Related Fatalities

FDA Data: 1990-1998

<u>Adverse Event</u>	<u>Cases</u>
Haemolysis	161 (50%)
Bacterial contamination	46 (10%)
TRALI	29 (9%)
Non-bacterial infections	23 (7%)
Transfusion-associated GvHD	18 (6%)

J-H Lee, MD, CBER, FDA, 9/24/99



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Death Blamed on Contaminated Blood!

Japan

The Asahi Shimbun, Wednesday,
September 3, 2003

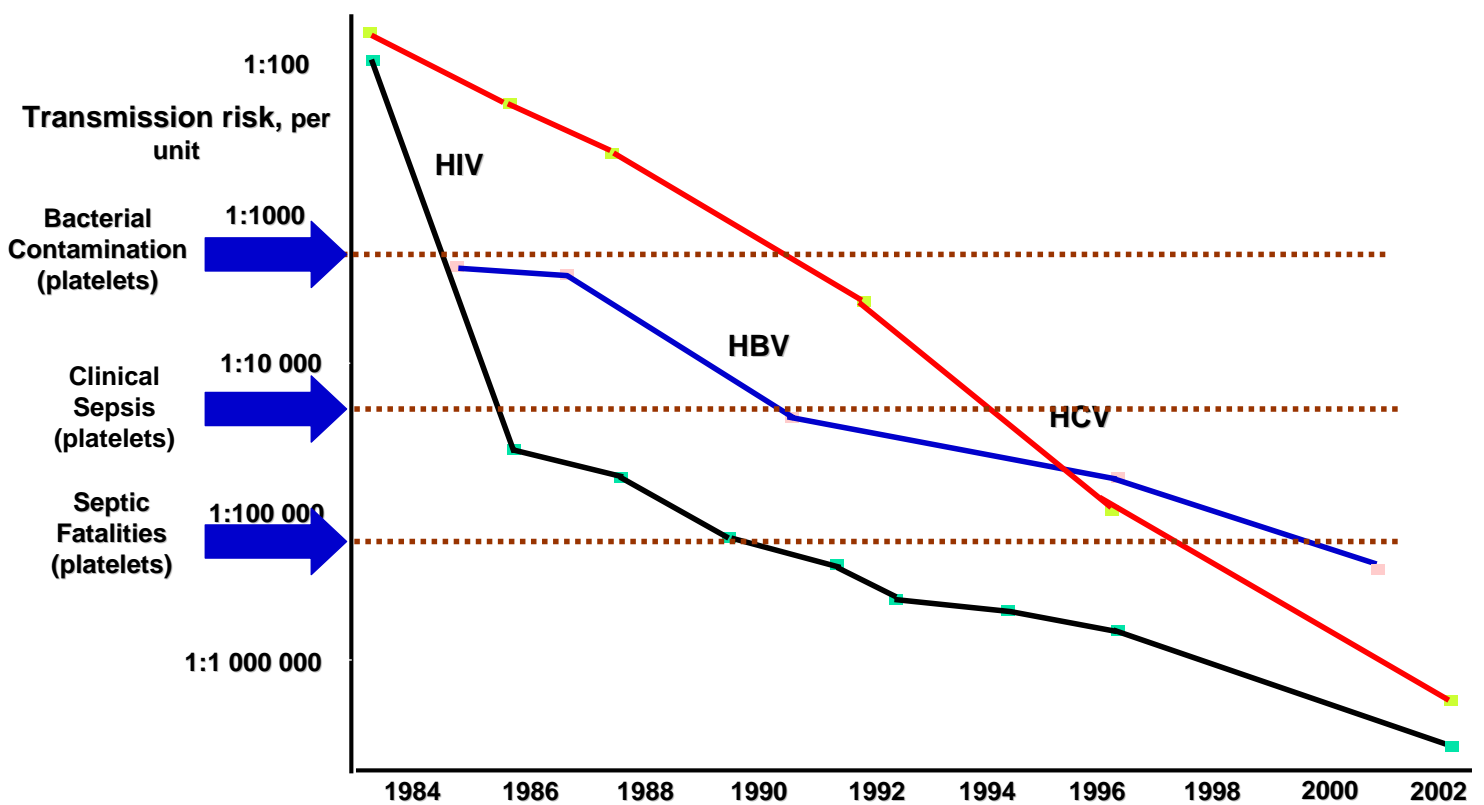
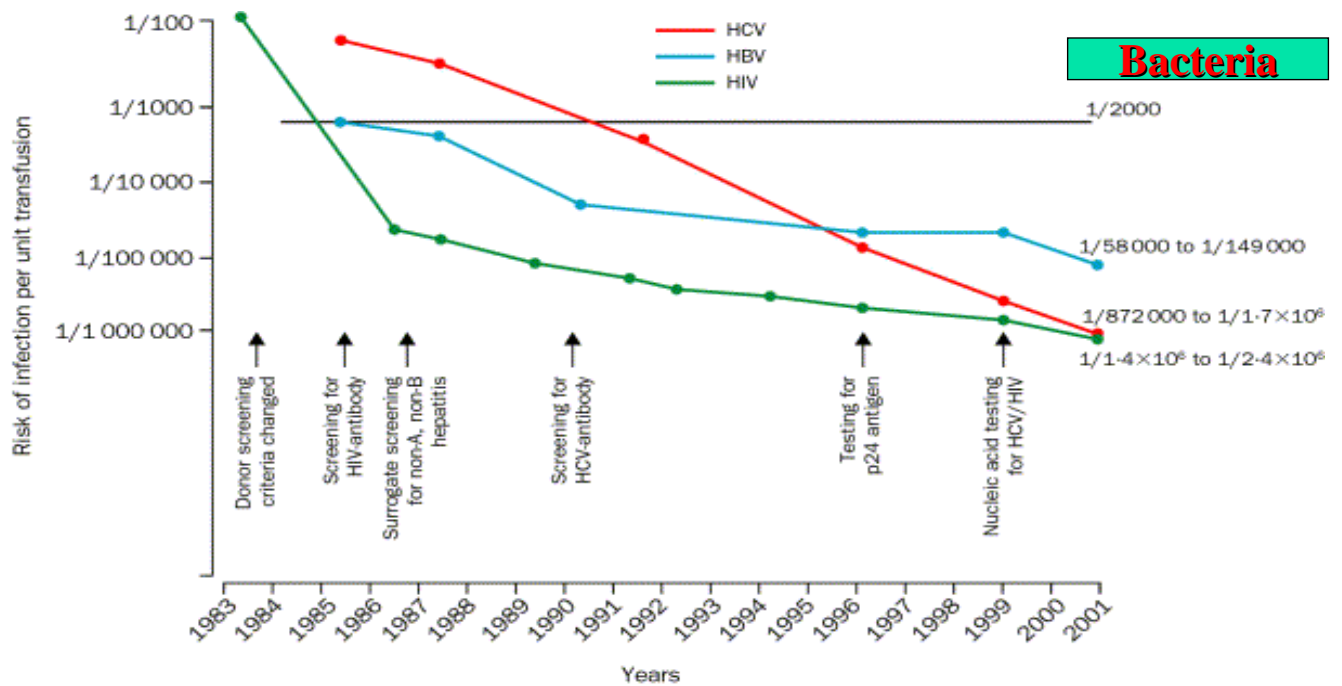
- Platelets, *Streptococcus pneumoniae*
- Male, died 9 hours after transfusion
- Same species 'also turned up in a different sample of the same donor'



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Residual risks of TTI



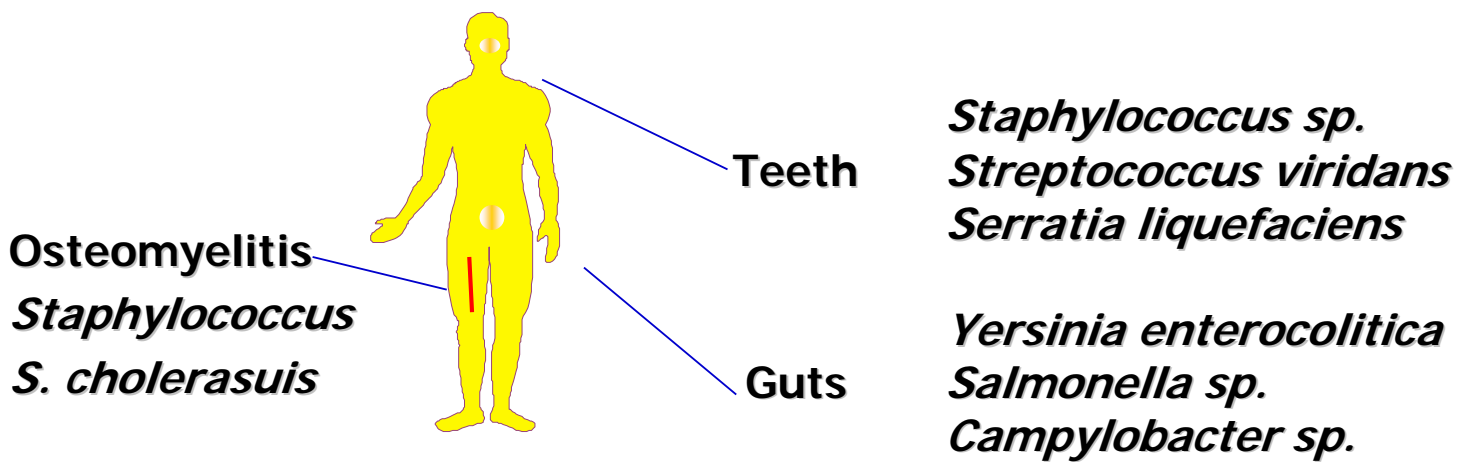
From a local clinical study, the risk of septic transfusion per unit of platelet concentrate is 1 in 2,000 (0.046%) among the BMT recipients

(A prospective study of symptomatic bacteremia following platelet transfusion and of its management
Chiu EKW et al. *Transfusion* 1994;**34**:950)

Contamination sources

- **Endogenous**
- **Exogenous**
- **Unknown**

Endogenous



Donor Screening

Exogenous

Normal Skin

Staphylococcus
epidermidis

Staphylococcus aureus

Diphtheroids sp.

Micrococcus sp.

Pseudomonas sp.

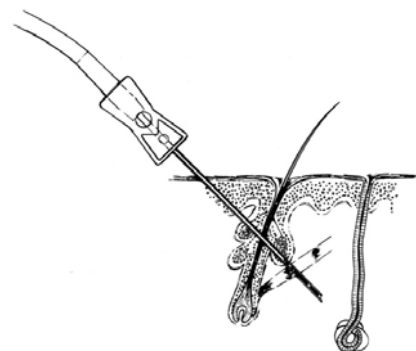
Bacillus cereus

Propionibacterium

acnes

Flavobacterium sp.

Exogenous



- Venipuncture standardization – Most important
- Contamination present in multiple puncture sites
- Dermic embolus – skin, follicles, sebaceous glands



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Exogenous

- Collection tubes ⇒ *Serratia marcescens*
- Non-sterile saline ⇒ Manipulation
- Water-bath - (*Burkholderia cepacea*, *P. aeruginosa*, *P. fluorescens*)



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Exogenous

- Contaminated bags - Scandinavia - *S. marcescens*
- Sterile connecting device - 1% failure
(Aubuchon et cols)
- Pinholes

Strategies to Decrease the Risk

- Donor Screening
- Improved Disinfection
- Diversion of the 1st Part of the Whole Blood Donation
- Bacterial detection
- Pathogen inactivation/reduction

Impact of donor skin preparation on the risk of bacterial contamination

CK Lee, PL Ho*, NK Chan, A Mak, J Hong, CK Lin. Hong Kong Red Cross Blood Transfusion Service and *Department of Microbiology, Queen Mary Hospital, the University of Hong Kong

Disinfectant used

- Method A
0.5% cetrimide/0.05% chlorhexidine solution followed by 70% alcohol (contact time for each was approximately 30 seconds)
- Method B
10% povidone-iodine followed by 70% alcohol (contact time for each was approximately 30 seconds)



Results

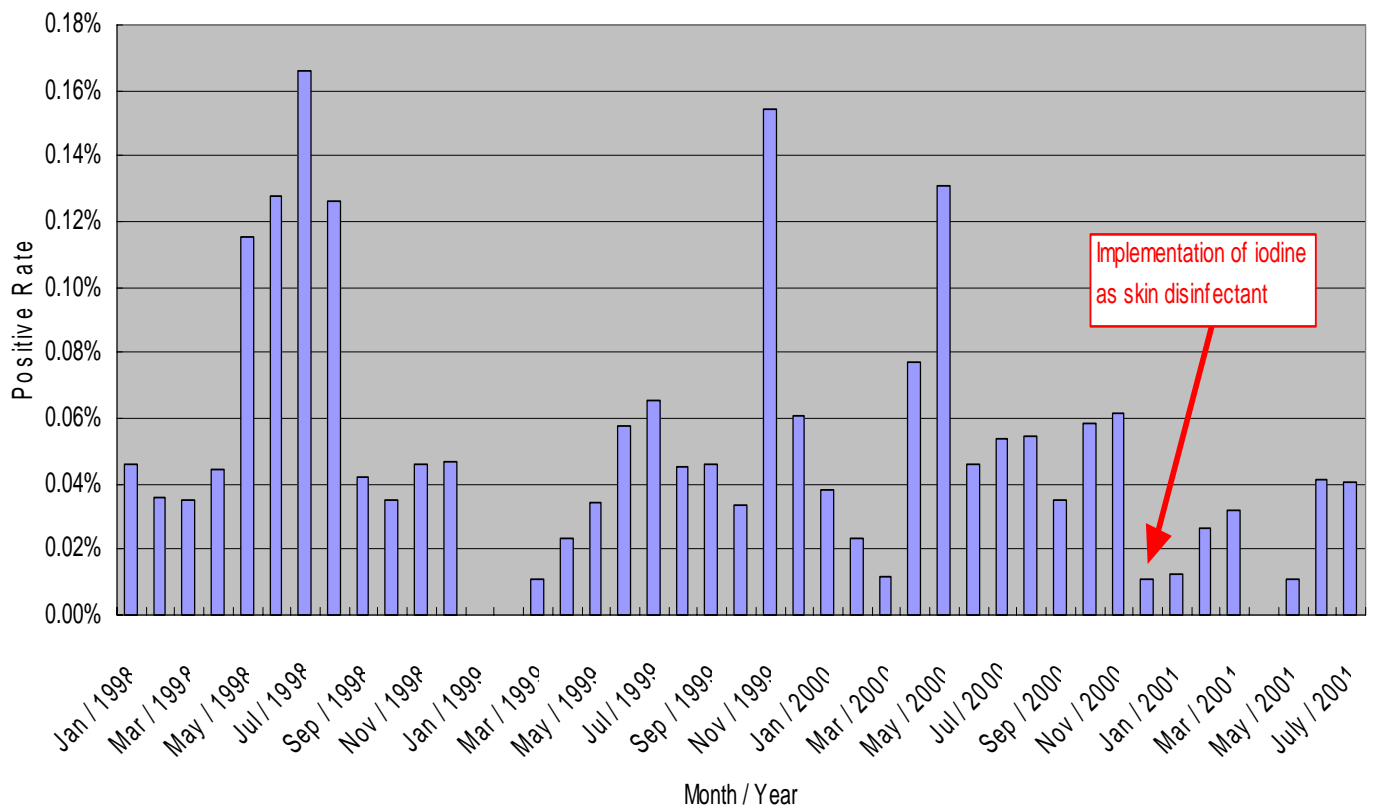
	Cetrimide/chlorhexidine, then alcohol	Povidone-iodine, then alcohol
No. of platelet concentrates examined	86558	86123
No. of platelet concentrates with bacterial contamination	62	36
Rates of bacterial contamination of platelet concentrates*	0.072%	0.042%

* $P = 0.0093$ by Chi-square test; relative risk reduction 41.7%.

Results: Micro-organisms identified

** $P = 0.7497$ by Chi-square test	Cetrimide/chlorhexidine, then alcohol	Povidone-iodine, then alcohol
<i>Bacillus species</i>	24 (38.7%)	16 (44.4%)
<i>Coagulase negative staphylococcus</i>	26 (41.9%)	15 (41.7%)
<i>Others: included Lancefield Group G Streptococci, Propionibacterium species, Diphtheroid bacilli, Proteus mirabilis</i>	12 (19.4%)	5 (13.9%)

Positive rate of bacterial surveillance test



Conclusion

- Povidone-iodine and alcohol is more effective than cetrimide/ chlorhexidine and alcohol in prevention of venepuncture-associated contamination of platelet concentrates by skin flora.
- However, it cannot completely eliminate contamination.

**Prevention: diversion of 1st
10 cc.**



Reduction of bacteria after diversion of first aliquot of blood

	Standard whole blood collection	Diversion of the 1 st 10 ml
Donations tested	18,257	7,115
Prevalence	0.34%	0.21%
Confidence interval	0.25-0.44	0.12-0.35

Reduced numbers of Staphylococci.

De Korte, Marcelis et al. 2000

CONCLUSIONS



- Prevalence of bacterial contamination in whole blood collections can be reduced significantly by removal of first amount of blood:
0.34% → 0.21%
- The theoretical contamination risk of pooled platelet concentrates composed out of 5 single donor units is still considerable: ~ 1%!
- **Screening platelets is recommended.**

US - AABB Standards

Date: March 3, 2003

To: AABB Members

From: Blood Bank/Transfusion Service Standards Program Unit

Re: Requirement for Implementation of Bacterial Detection Methods

5.1.5.1 The blood bank or transfusion service shall have methods to limit and **detect bacterial contamination in all platelet components.**

Standard 5.6.2 applies. [Arm Prep]

5.1.5.1 Standard 5.1.5.1 shall be implemented by **March 1, 2004.**

CAP

College of American Pathologist's has included Bacterial contamination of Platelets in their Accreditation Checklist (December, 2002):

“TRM.44955 Phase 1 Does the laboratory have a system to detect the presence of bacteria in platelet components?”

Reduction of platelet transfusion- associated sepsis by short-term bacterial culture Liu HW et al. *Vox Sang* 1999;77:1-5

- 26,210 whole-blood-derived platelet components were tested by aerobic bacterial culture on day 2.
- 14 (0.053%) platelet units were found to be contaminated.
- nine of the associated red cell units and 4 fresh-frozen plasma units grew the same organisms on culture.
- Risk of bacterial contamination: 1:1872



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



HK Red Cross Blood Transfusion Service implemented a program of pre-release bacterial surveillance for platelet concentrates since January 1998



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



A total of 543,819 units of
platelets have been cultured
(up to end of January 2003)

Test Method

Sample

- Platelet concentrates prepared from whole blood units which have been stored at RT for 6 to 24 hours,
- 1 to 1.5 ml from each platelet unit on D2,
- Samples from 5 units pooled into one for culture.

Test Method

Reagent & Equipment

- BacT/Alert aerobic culture bottles
- BacT/Alert Automated Microbial Detection System

Test Method

Procedure

- Inoculate the pooled samples into aerobic culture bottles,
- Incubate for 24 hours at 35°C,
- If negative, platelets can be issued,
- Further incubate for 24 hours.

Platelet concentrates on Day 2 of collection



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Strip and mix the PRP in tubing and bag



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Seal the tubing segment at the required length



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Fasten the 5 tubing segments together



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Assign an accession number to each member of the platelet bag and tubing bundle in one pool



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



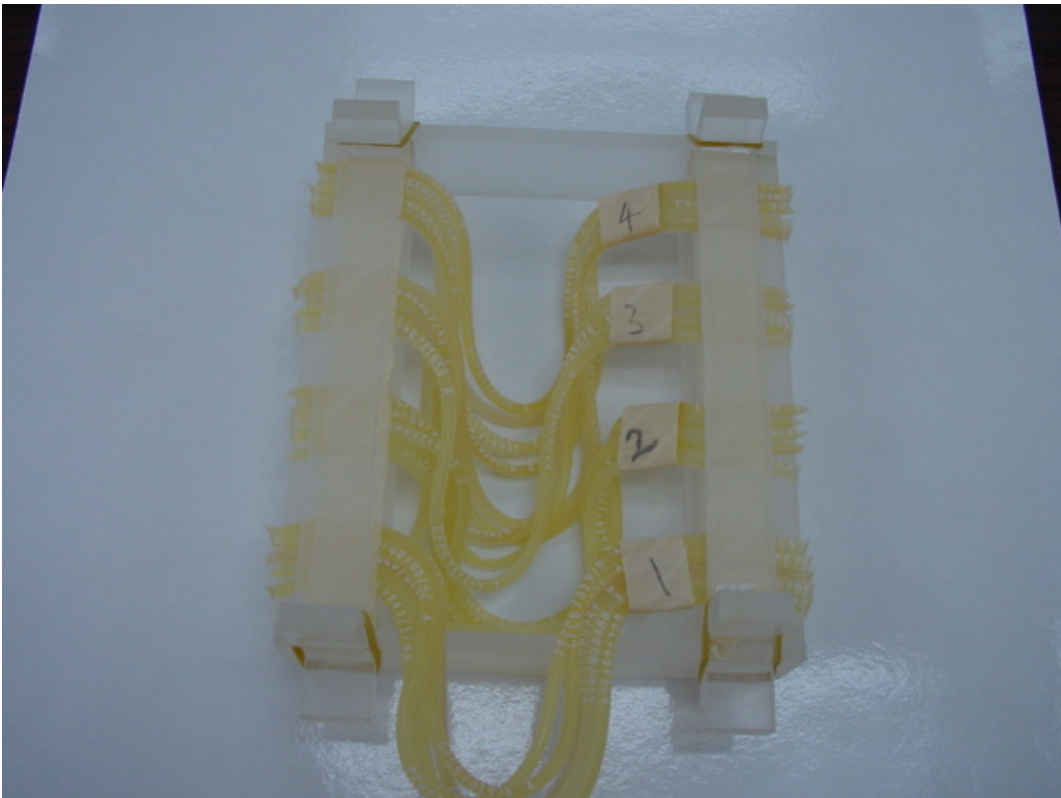
Cut the segments out



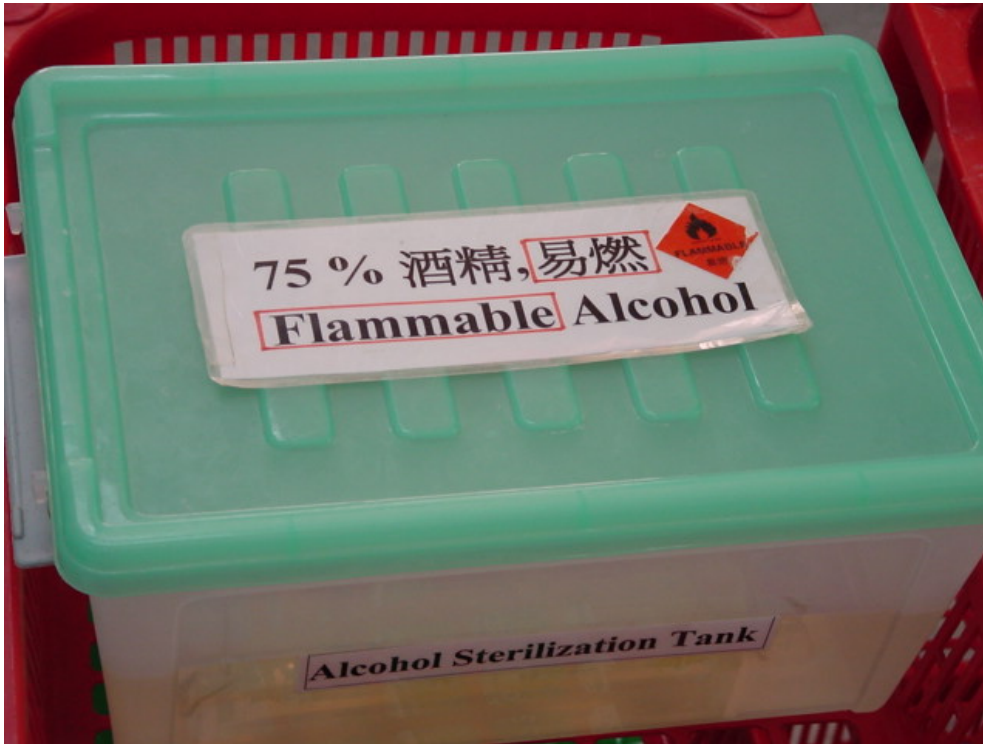
Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Fit the bundle of segment into a rack



Immerse the segment bundles with the rack into alcohol for 10 min for sterilization

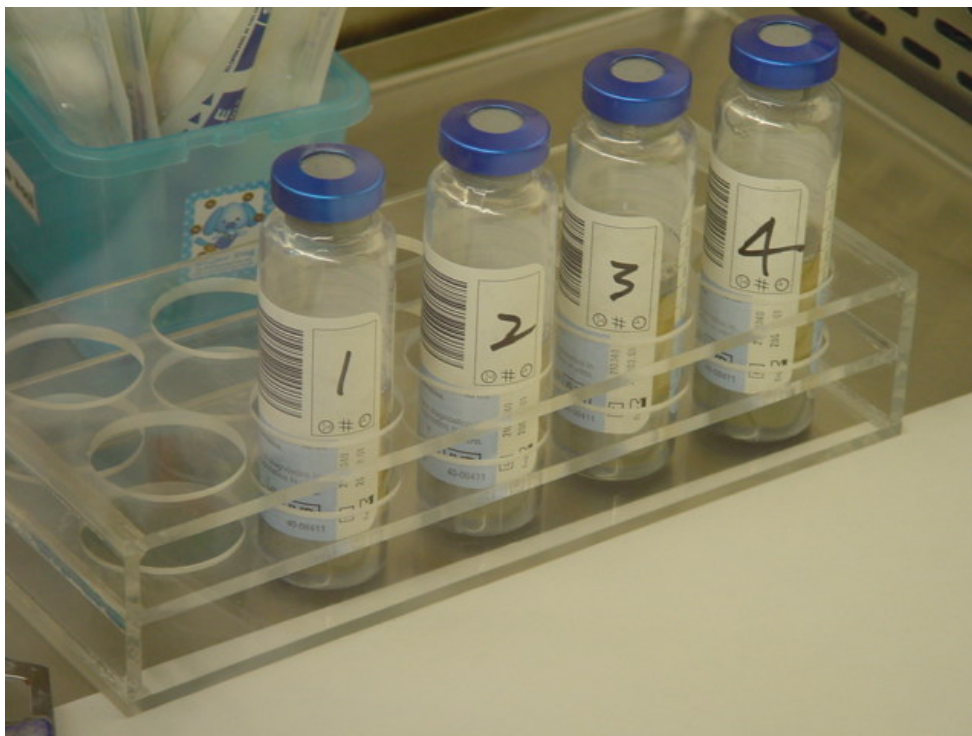


 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Prepare the aerobic culture bottle



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority

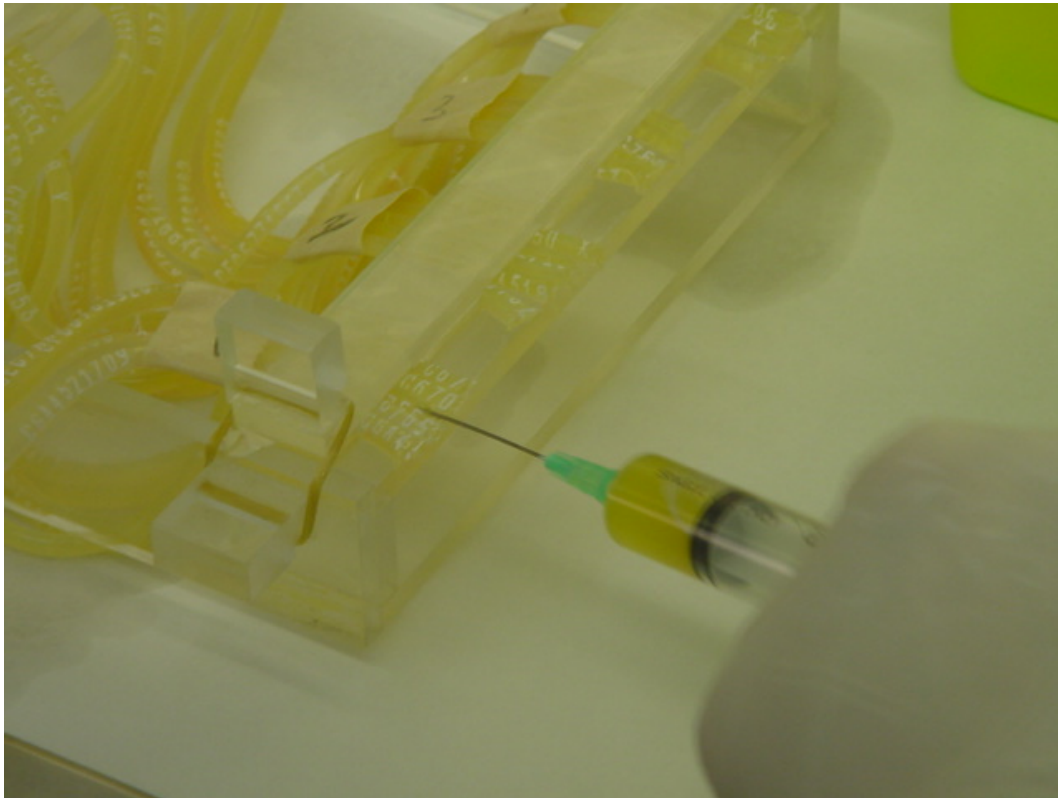


Aspirate sample from tubing segments with syringe



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority





Inoculate into culture bottle



Register bottle for loading into the detection system



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Load the bottle into the detection system



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Initial report printed after first 24 hours of incubation



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Release platelet if result is negative after the first 24 hours



 Hong Kong Red Cross Blood Transfusion Service, Hospital Authority 

Final report printed after full 48 hours incubation



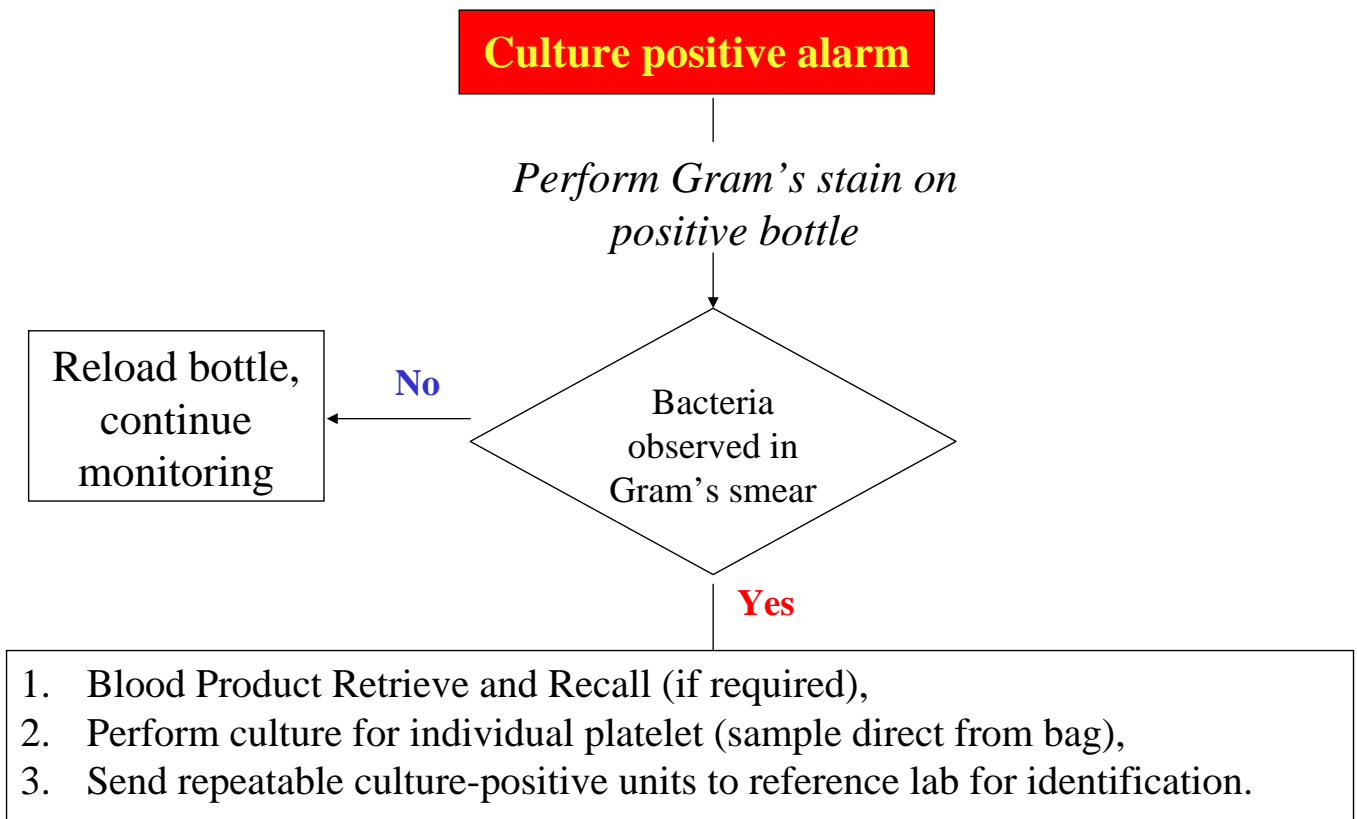
 **Hong Kong Red Cross Blood Transfusion Service, Hospital Authority** 

Initial positive rate

0.47% per pool

 **Hong Kong Red Cross Blood Transfusion Service, Hospital Authority** 

Follow up of initial positive samples

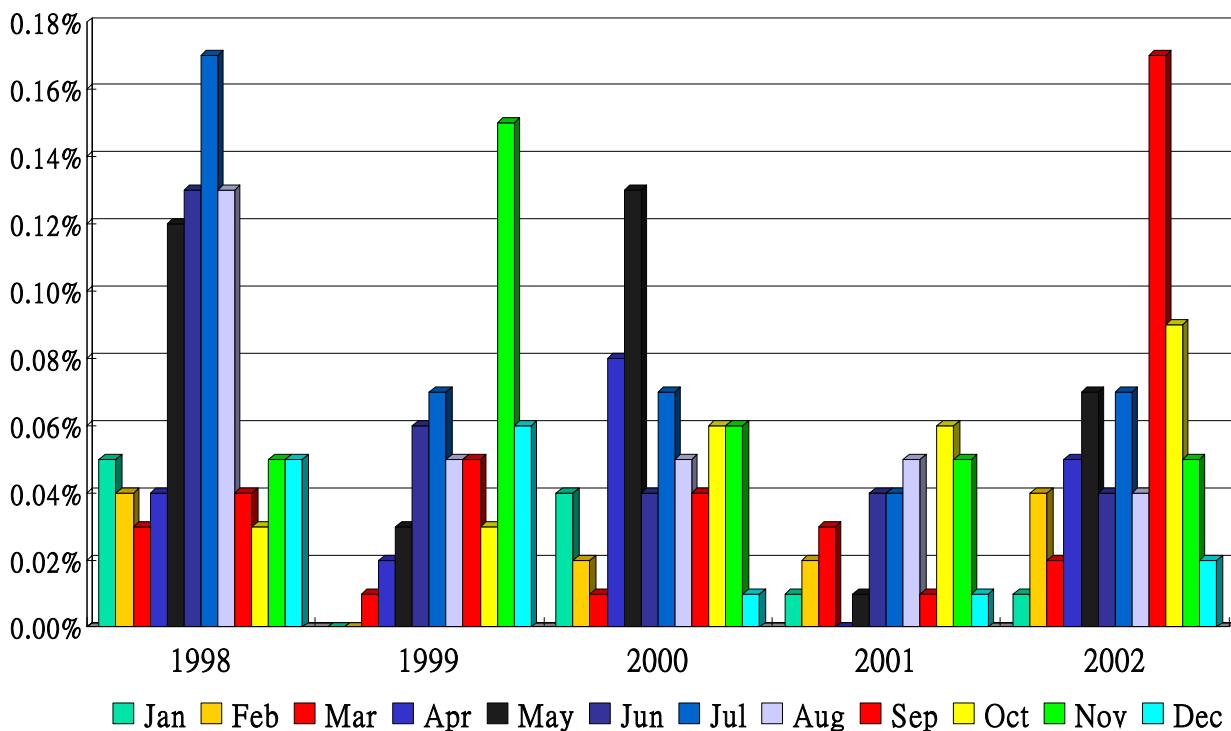


Confirmed Positive Rate

- Per pool: 0.25% (53% of the initial positive pools)
- Per unit of platelet: 0.0519% (1/ 1927 units)

Bacterial surveillance positive rate

细菌监测实验阳性率



Confirmed positive cases

Among the 282 confirmed positive cases:

- Bacillus spp: 151 (53.5%)
- Coag-neg Staph: 93 (33%)
- Misc Gram positive org: 14 (5%)
- Misc Gram negative org: 24 (8.5%)

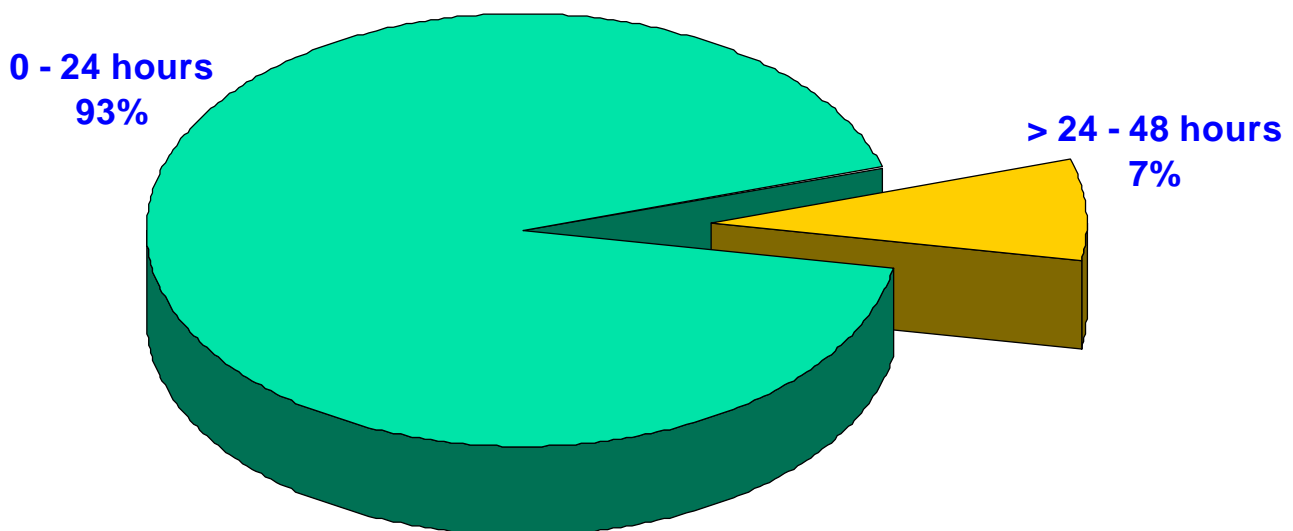
Misc Gram +ve organisms include:

- Lancefield Group G streptococci
- Non-enterococcal Group D streptococci
- Micrococcus spp

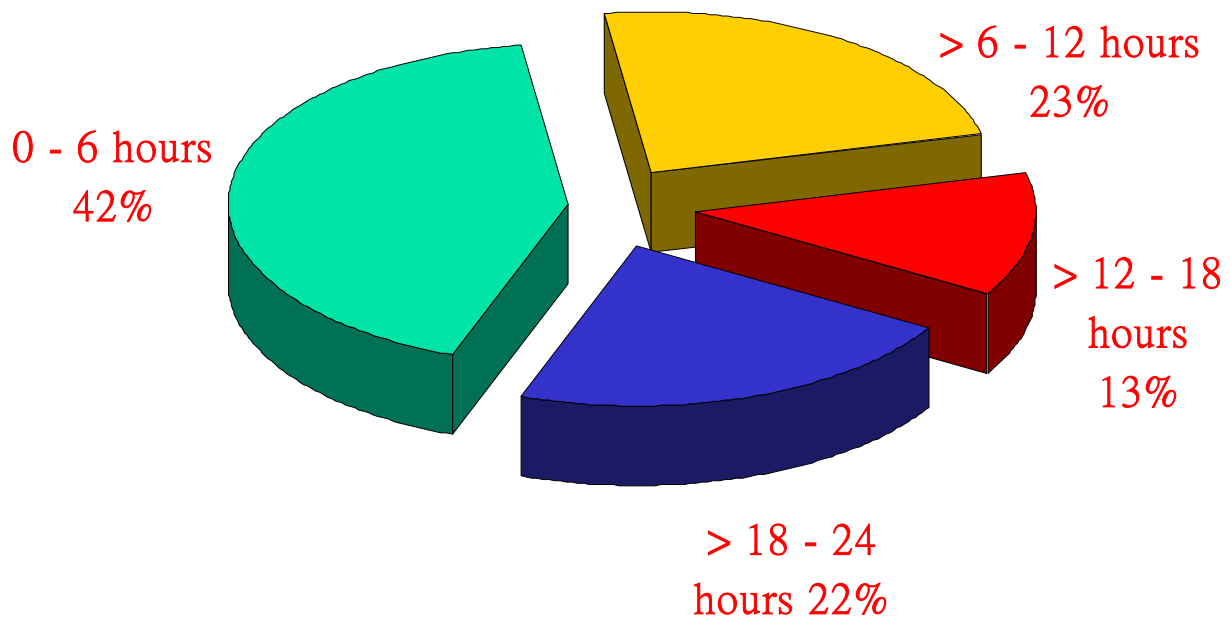
Misc Gram –ve organisms include:

- E coli
- Klebsiella oxytoca
- Proteus mirabilis
- Salmonella group D
- Flavobacterium spp
- Haemophilus influenzae

Breakdown of positive samples by detection time



Breakdown of positive samples (within 24 hours) by detection time



Recall of platelets from hospitals

- 173 units recalled because of initial positive
- 6 (3.5%) confirmed positive
- 3 (50%) due to Bacillus
- 3 (50%) due to CNS

Conclusion

1. Test simple and not expensive;
2. There has been no documented report of septic platelet transfusion since introduction of BST in 1998 in HK;
3. Effective in eliminating the risk of sepsis due to bacterial contamination

Disadvantage

1. Platelets are normally released at Day 3 of collection \Rightarrow shorten the available shelf life;
2. One positive platelet will cause wastage of other platelets in the pool.

Residual Risk of Bacterial Contamination Of Platelet Concentrates At The End Of Shelf Life (Day 5 & 7) After Routine Bacterial Surveillance At Day 2

Estimation of bacterial risk in extending platelet concentrates shelf life from 5 to 7 days

Objectives of study

- To quantify the residual risk of bacterial contamination of platelet concentrates at the end of their shelf life (5 and 7 days) by culture method after routine bacterial surveillance testing (BST) at day 2,
- To identify the nature of bacteria involved.



Protocol

Day 0	Blood collection
Day 1	Component preparation
Day 2	Bacterial culture for 24 hours (BST)
Day 3	Release of BST negative PC, continue to monitor BST for another 24 hours
Day 4	BST completed
Day 5	PC expires
	50% expired PC → bacterial culture on expiry
	50% expired PC → bacterial culture on Day 7



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Results(1)

	<u>Group A (PC stored for 5D)</u>	<u>Group B (PC stored for 7D)</u>
Total no. of samples studied	3010	3010
No. of initial but not confirmed positive cultures	1	2
Total no. of confirmed positive cultures	4	4
Overall positive rate	0.133%	0.133%



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Results(2)

	Case	Gram Stain	Bacteria identified	Time to detect	Culture of associated blood products
A	S731	+ve	<i>Coagulase Negative Staphylococcus</i>	4.2 hours	RC (-), FFP (-)
	SX290	-ve	<i>Propionibacterium acnes</i>	6.6 days	RC (+), FFP (-)
	S1222	-ve	<i>Propionibacterium acnes</i>	5.3 days	RC (+), FFP (-)
	SX1046	-ve	<i>Propionibacterium acnes</i>	5.8 days	RC (+), FFP (-)

Results(3)

	Case	Gram Stain	Bacteria identified	Time to detect	Culture of associated blood products
B	EX201	+ve	<i>Coagulase Negative Staphylococcus</i>	5.7 hours	RC (-), FFP (-)
	EX565	-ve	<i>Propionibacterium acnes</i>	6.5 days	RC (+), FFP (-)
	E1491	-ve	<i>Propionibacterium acnes</i>	5.5 days	RC (T), FFP (-)
	E2042	-ve	<i>Coagulase Negative Staphylococcus</i>	10.7 hours	RC (-), FFP (T)

Conclusion(1)

- Bacterial contamination is still present in platelet concentrates at 5 days or 7 days from collection despite routine bacterial surveillance (BST) on day 2.
- The risk bacterial contamination at day 5 or 7 after BST is about 0.133%.
- *Propionibacterium acnes* and *coagulase negative Staphylococcus* are the bacteria identified.

Conclusion(2)

- These bacteria were not identified in the BST culture bottles when incubated for 7 days (because of sampling effect or the original bacterial load being too low).
- *Propionibacterium acnes* is believed to be clinically insignificant because of its slow growing nature and the lacking of report of clinically significant sepsis.
- If *Propionibacterium acnes* is regarded as clinically insignificant, the residual risk will be reduced to 0.033% and 0.066% at day 5 & 7 respectively.

Conclusion(3)

- We therefore cannot justify the application of BST to extend platelet shelf life from 5 to 7 days without additional risk of bacterial contamination.



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Pathogen Inactivation/Reduction- Major Concerns

- Platelet Loss up to 30%
 - Processing
 - Chemical Compound
- Killing Capacity:
 - Unable to Kill HAV
 - Log 4 – 5 Bacillus, Pseudomonas, Not Effective Against Bacterial Spores
 - In General log 6 up to 8 for Viruses
 - Viremic Stage: Higher Number of Particles (10^{12} Parvo B19, 10^7 - 10^8 for HIV – HCV even in the Chronic Infection Stage)



Hong Kong Red Cross Blood Transfusion Service, Hospital Authority



Pathogen Inactivation/Reduction- Major Concerns

■ Cost-Effectiveness

- Viral and Bacterial Risk Reduced by Testing
- Price-Positioning ~ 120 – 100 USD per Product
- Labour Intensive, Space Occupying

■ Potential Side Effects

- Toxicity
- Mutagenicity



*Thank you for
your attention!*